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Studies on fungi in a pine-wood soil
II. Substrate relationships of fungi in the mineral horizons of the soil(1)

BY

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Introduction:

The application of the soil washing technique in studies on soil fungi (as described previously, PARKINSON and BALASOORIYA, 1967) allows information to be obtained on the relationships of the various fungi to specific groups of microhabitats in the soil. The technique allows a fractionation of the soil samples into discrete substrate units which can be picked out individually and plated onto nutrient agar media. Thus it is easily possible to separate organic matter from mineral particles, and the mineral grains may be separated on a particle size basis on the sieves in the washing boxes.

In the present investigation the application of the soil washing technique yielded only two distinct types of washed material — small root fragments and mineral particles (0.25-0.5 mm). A detailed study has been made of the amount and type of fungal colonization of these two distinct groups of microhabitats.

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Method.

The method for isolating fungi from this soil has been described previously (PARKINSSON and BALASOORIYA, 1967). The soil samples (from the A_1 and C horizons of the Freshfield soil) used for this investigation were the same as those used in the previous investigation (PARKINSON and BALASOORIYA, 1967) — the two investigations being performed concurrently.

Results and Discussion.

Of a total of 39 fungal species isolated from the A_1 and C horizons of the Freshfield soil, 20 were found to be isolated from both organic matter and mineral particles, 16 were restricted to organic fragments, and 3 were restricted to mineral particles. These 3 groups of fungi are listed in Table I.

TABLE I

Fungi associated with mineral and organic particles

Fungi associated with both mineral and organic particles	Fungi restricted to organic particles	Fungi restricted to mineral particles
Aspergillus sp.	*Mortierella marburgensis*	*Chaetosphaeria myriocarpa*
Absidia coerulea	*Mortierella parvispora*	*Gliomastix gutuliformis*
Cylindrocarpon cylindroides	*Mucor hiemalis*	
Cylindrocarpon radicicola	*Paecilomyces carneus*	*Zygorrhynchus moelleri*
Gliomastix murorum	PCB 10	
Mortierella alpina	*Phialophora* sp.	
Mortierella minutissima	*Phoma* sp. 1	
Mortierella vinacea	*Phoma* sp. 2	
Oidiodendron fuscum	*Phoma* sp. 3	
Penicillium 101	*Phoma* sp. 4	
Penicillium citrinum	Sterile dark form (AR10)	
Penicillium decumbens	Sterile dark form (AR11)	
Penicillium lividum	Sterile dark form (BR2)	
Phialophora fastigiata	Sterile dark form (GRN)	
Sterile hyaline form (HS)	Sterile dark form (GTGN)	
Sterile hyaline form (W2)	*Verticillium nigrescens*	
Sterile dark form (CR3)		
Trichoderma viride		
Verticillium sp. 2		

The small number of species restricted to mineral particles (plus the very sporadic nature of their isolation) might be expected because of the comparatively small size of these particles and because the majority of the particles possessed no coating of humic material (which might afford nutrients). However, microscopic examination of washed mineral particles often showed the presence of pieces of mycelium attached to them.

Decomposing organic particles provide the major loci of fungal development in any soil, and in the skeletal soil under investigation have this phenomenon, was very apparent. Certain fast growing fungi (e.g. *Mortierella*

parvispora and *Mucor hiemalis*) were restricted to organic fragments, but in the main it was slower growing organisms which were isolated solely from these fragments. The ability of these slow growing forms to complete efficiency for substrates with the more widespread and rapidly growing forms (e.g. *Trichoderma viride* and *Mortierella* spp.) is difficult to explain.

Amongst the sterile isolates which were found to be confined to dead root fragments, sterile dark from (GRN) was of particular interest. Direct observation of washed root fragments showed the presence of a dark, septate mycelium, morphologically similar to this isolate, attached to them. Comparison of this isolate with the *Phialophora* sp. isolated (WILLIAMS, 1963) from the Delamere forest soil showed great similitaries between the two isolates, however attempts to induce spore production in the isolate from the Freshfield soil met with no success. Again, this sterile dark form (GRN) appears to be very similar to some cultures of *Mycelium radicis atrovirens*. The regular association of sterile dark form (GRN) with decomposing root fragments and its regular isolation from the stele of living roots of *Pinus nigra* (Hodkinson, pers. comm.) point towards an intimate association of this fungus with roots of *Pinus nigra*. It may well be that these slow growing species of poor competitive ability for dead organic material were found on the organic fragments (which, as stated earlier, were root fragments) because they had colonized this material when it was part of a living root system i.e. they represent a root surface or pseudomycorrhizal population which with death of the roots, has be come an agent of root decomposition.

Of the fungi isolated from both organic and mineral particles *Trichoderma viride*, *Cylindrocarpon radicicola*, and *C. cylindroides* showed a markedly increased frequency of occurrence on organic fragments (the distribution of these species on organic and mineral particles from the mineral horizons is shown in Table II), whilst *Penicillium decumbens* and Penicillium 101 (believed to be an hitherto undescribed species showed a clear affinity for mineral particles (see Table II).

TABLE II

Percentage frequency of occurrence of certain fungi on mineral and organic particles (from the A and C horizons of the Freshfield soil)

FUNGI	A		C_1		C_2	
	organic	mineral	organic	mineral	organic	mineral
Cylindrocarpon cylindroides	5.0	0	10.0	5.0	5.0	0
Cylindrocarpon radicicola	5.0	2.5	27.5	2.5	32.5	0
Trichoderma viride	27.5	12.5	12.5	10.0	7.5	2.5
Penicillium decumbens	30.0	55.0	25.0	40.0	9.0	16.0
Penicillium 101	1.0	3.5	1.0	2.5	0	1.5

The over-all fungal development of any soil horizons, as demonstrated by the soil washing technique, may be considered as proportional to:

a) the total number of particles colonized by fungi;

b) the average number of colonies developing from each plated particle.

The product of these two values gives a composite figure which can be considered as an index of fungal development, and is subsequently referred to as the « colonization index ».

Table III indicates the colonization of index of each horizon in the Freshfield soil (A_0, A_1, C) from this it can be seen that in the layers of the A_0 horizon the colonization index progressively increases with depth to the H horizon. Thereafter, in the mineral horizons, there was a decrease in colonization index with depth. Reduction in the extent of fungal development with depth in mineral soil has been observed by numerous workers (e.g. SEWELL, 1959; BROWN, 1958; WILLIAMS, 1962), MCLENNAN and DUCKER (1954), on the other hand, observed a decrease in overall « activity » with the decrease in total organic matter but not with depth. In the Freshfield soil the amount of organic matter decreases with depth — and the decreased fungal development may be due to one or other or both these factors.

TABLE III

Horizon	% Particles Colonized	Av. No. of Colonies/Particle	Colonization index
L	70.3	0,91	63.97
F_1	79.3	1.19	94.37
F_2	94.0	1.67	156.98
H	95.4	1.89	180.31
A	69.7	0.78	54.37
C_1	45.3	0.48	21.74
C_2	23.1	0.24	5.54

When evaluating the overall fungal development in the mineral horizons, of the Freshfield soil it is important to note that the overall values have been obtained (Table III) by calculation of the mean from an equal number of washed mineral and organic particles plated. Therefore these values would only be truly representative if the soil horizons contained more or less equal amounts of mineral and organic matter (the importance of this was stressed by WILLIAMS, 1962). As the mineral horizons of the Freshfield soil contain much less than 50 % organic matter the data provided are distorted, however it is felt that the values in Table III give a useful (if crude) comparative indication of fungal development in these horizons.

Table IV gives data on this colonization index for organic and mineral particles in the A_1 and C horizons of the Freshfield soil. From these data it can be seen that, as expected, organic fragments had higher colonization indices than mineral particles (although the values for the two types of particles were in the main, similar).

WILLIAMS (1962) found, working with soil from the Delamere forest podzol, that the colonization index of mineral particles was considerable lower than that of organic fragments. The higher degree of colonization of

TABLE IV

Overall colonization of organic and mineral particles from the mineral horizon of the Freshfield soil

	A		C_1		C_2	
	organic	mineral	organic	mineral	organic	mineral
% particles colonized	70.0	69.4	46.3	43.6	32.6	17.4
Average number of colonies/particle	0.87	0.72	0.48	0.47	0.34	0.18
Colonization Index	60.9	49.9	22.2	18.5	11.1	3.1

mineral particles in the Freshfield soil is perhaps an effect of the presence of *Penicillium decumbens* and *Penicillium* 101 which, as stated earlier, appear adapted for growth on mineral particles. The sparse distribution of organic fragments in the soil may also necessitate active spread of organisms through the mineral matrix.

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